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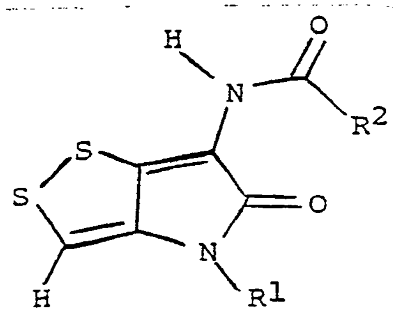
(54) Title: XENORHABDIN ANTIBIOTICS

(I)



Xenorhabdin I	R^1_1 = H,	R_2 = n-pentyl
Xenorhabdin II	R^1_1 = H,	R^2_2 = 4-methylpentyl
Xenorhabdin III	R^1_1 = H,	R^2_2 = n-heptyl
Xenorhabdin IV	R^1_1 = CH_3	R^2_2 = n-pentyl
Xenorhabdin V	R^1 = CH_3;	R^2_2 = 4-methylpentyl

(57) Abstract

The new 1,2-dithiolo[4,3-b]pyrrole derivatives (also designated as Xenorhabdins) of formula (I): Xenorhabdin I: R^1 = H, R_2 = n-pentyl; Xenorhabdin II: R' = H, R_2 = 4-methylpentyl; Xenorhabdin III: R' = H, R_2 = n-heptyl; Xenorhabdin IV: R' = CH_3, R_2 = n-pentyl; Xenorhabdin V: R^1 = CH_3, R_2 = 4-methylpentyl are antibiotics and pesticides. Their bio-syntheses from Xenorhabdus nemtophilus or X. luminescens, and the pharmaceutical and pesticidal formulations containing them have been disclosed. Several xanthydrol and acetate derivates of Xenorhabdins have been prepared.

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"XENORHABDIN ANTIBIOTICS"

TECHNICAL FIELD

This invention relates to antibiotics and pesticides, and in particular to antibiotic substances and pesticides which may be isolated from micro-organisms associated with certain nematodes.

BACKGROUND ART

Insect pathogenic nematodes of the families **Heterorhabitidae** and **Steinernematidae** are known to be symbiotically associated with bacteria of the genus **Xenorhabdus**, and it has been observed that these bacteria have the ability to inhibit the activity of other bacterial genera. **Xenorhabdus** are described by Thomas and Poinar, Int J. Syst. Bacteriol., 29 (4), 352-360, (1979), Akhurst, Int. J. Syst. Bacteriol., 33 (1), 38-45, (1983), Akhurst, J. Gen. Microbiol., 121, 303-309, (1980), and Akhurst, J. Gen. Microbiol., 128, 3061-3065, (1982). The activity is believed to derive from antibiotic substances released by the **Xenorhabdi**. Paul et al. (**J. Chem.Ecol**, 7(3), 589-594 (1981)), for example, have confirmed the presence of certain indoles and stilbenes in cultures of **X.nematophilus** and **X.luminescens**, and have shown that these compounds are active against a number of non-pathogenic, bioluminescent bacteria.

Paul **et al.** suggest that several antibacterial mechanisms may be operating in the **Xenorhabdus** systems. This possibility could explain the fact that we have succeeding in isolating from **X.nematophilus**, antibiotic compounds of quite different structure from those investigated by Paul et al. The new compounds have, moreover, proven to be active against a wide range of bacteria, including Gram positive species and are also effective as pesticides, especially as insecticides.

Bacteria of the genus **Xenorhabdus** are found to occur in two forms, called primary (1^o) and secondary (2^o) form. The two forms of **X norhabdus** can be differentiated most easily by their colonial morphology. Antibiotic activity is exhibited by primary form bacteria, but not by

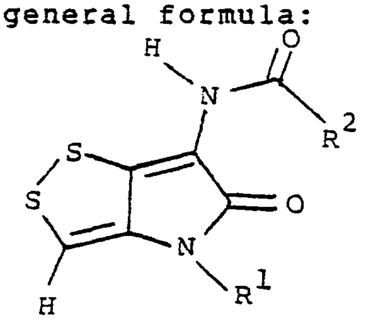
secondary forms.

DISCLOSURE OF INVENTION

Some of the compounds of this invention, which were isolated from primary form **X.nematophilus** strain T319 (Enterobacteriaceae), are characterized in that they yield a peak due to ions of elemental composition $C_5H_4N_2OS_2$ in their 70eV electron ionisation mass spectra, and that the collision-induced dissociation spectrum ('Collision Spectroscopy', Plenum Press, New York 1978, Ed., R.G. Cooks) of these ions is that depicted in Fig. 1. The collision-induced dissociation spectrum may be obtained by scanning a VG Micromass 70/70 mass spectrometer at a constant ratio of the magnetic field to electric sector voltage, with helium in its collision cell at an estimated pressure of 0.04Pa.

The compounds of the invention exhibit antibiotic and pesticidal activity. Some of the compounds are 1,2-dithiolo[4,3-b]pyrrole derivatives, whilst others are as yet unidentified. Compounds of the invention are either isolated from **X.nematophilus** or **X.luminescens**, or derived from compounds so isolated.

The 1,2-dithiolo[4,3-b]pyrrole derivatives may be represented by the general formula:



(I)

These compounds of the invention have been called "xenorhabdins". The xenorhabdins isolated from **X.nematophilus** are as follows:

Xenorhabdin I	R^1 = H, R_2 = n-pentyl
Xenorhabdin II	R^1 = H, R_2 = 4-methylpentyl
Xenorhabdin III	R^1 = H, R_2 = n-heptyl
Xenorhabdin IV	R^1 = CH_3, R_2 = n-pentyl
Xenorhabdin V	R^1 = CH_3, R_2 = 4-methylpentyl

Salts, acyl and other derivatives of the xenorhabdins and other compounds of the invention also form part of the present invention.

Thus the xenorhabdin compounds of the invention are 6-hexanoylamino-1,2-dithiolo[4,3-b]pyrrol-5-(4H)-one 6-(5-methyl-hexanoylamino)-1,2-dithiolo[4,3-b] pyrrol-5-(4H)-one, 6-octanoylamino-1,2-dithiolo[4,3-b]pyrrol-5(4H)-one, 6-hexanoylamino-4-methyl-1,2-dithiolo[4,3-b]pyrrol-5(4H)-one and, 6-(5-methylhexanoylamino)-4-methyl-1,2-dithiolo[4,3-b] pyrrol-5(4H)-one.

The invention also includes processes for the preparation of the aforesaid antibiotic and pesticidal compounds, which, inter alia, may be isolated from a natural source, such as cultures of **Xenorhabdus**, in a manner known per se, for example by extraction with an organic solvent such as ethanol, DMSO, ethyl acetate or chloroform, and subsequent chromatography, preferably with a silica gel stationary phase.

Thus the invention also provides a process for the production of xenorhabdins I, II, III, IV or V, which process comprises culturing a xenorhabdin producing strain of **Xenorhabdus nematophilus** or **Xenorhabdus** luminescens in a suitable culture medium and separating the xenorhabdin I, II, III, IV or V from the resultant culture broth.

Also included within the invention is a process for the production of other compounds having antibiotic and/or pesticidal activity, which process comprises culturing a xenorhabdin producing strain of **Xenorhabdus nematophilus** or **Xenorhabdus luminescens** in a suitable culture medium and separating the other compounds having antibiotic and/or pesticidal activity from the resultant culture broth.

In a further aspect, the invention provides a continuous process for the production of xenorhabdins I, II, III, IV or V, which process comprises culturing a xenorhabdin producing strain of **X norhabdus nematophilus** or **Xenorhabdus lumin scen** in a suitable culture medium in a fermenter in the presence of oxygen, controlling the temperature and pH of the culture in said fermenter,

continuously adding fresh culture medium to said fermenter and continuously collecting culture from said fermenter at such a rate to maintain the volume of culture in said fermenter within selected limits, and separating xenorhabdins I, II, III, IV and V from the collected culture.

The invention also provides a continuous process for the production of other compounds having antibiotic and/or pesticidal activity, which process comprises culturing a xenorhabdin producing strain of **Xenorhabdus nematophilus** or **Xenorhabdus luminescens** in a suitable culture medium in a fermenter in the presence of oxygen, controlling the temperature and pH of the culture in said fermenter, continuously adding fresh culture medium to said fermenter and continuously collecting culture from said fermenter at such a rate to maintain the volume of culture in said fermenter within selected limits, and separating the other compounds having antibiotic and/or pesticidal activity from the collected culture.

In yet another aspect, the invention also provides pharmaceutical formulations which comprise at least one compound of formula I **supra** or other compound of the invention having antibiotic activity, together with a pharmaceutically acceptable carrier or diluent therefor.

Also included within the scope of the invention are pesticidal formulations which comprises at least one compound of formula I **supra** or other compound of the invention having pesticidal activity, together with a carrier or diluent therefor.

The invention also provides a method for the prevention or control of infectious disease in a mammal requiring said prevention or control, which method comprises administering to said mammal an effective amount of at least one compound of formula I **supra** or other compound of the invention having antibiotic activity, or of a formulation containing same.

The invention further provides a method of killing or controlling pests at a locus at which said pests occur or are expected to occur which method comprises applying to

said locus an effective amount of at least one compound of formula I supra or other compound of the invention having pesticidal activity, or of a formulation containing same.

MODES FOR CARRYING OUT THE INVENTION

Suitable culture media include materials containing suitable carbon and energy sources, such as glucose or other sugars, glycerol, or lipids, suitable nitrogen sorces such as ammonia, urea, amino acids, peptides or proteins, appropriate quantities or inorganic nutrients such as phosphate, potassium, magnesium, calcium and trace elements, and some source of vitamins and growth factors, e.g. yeast extract.

Such a medium used and found suitable for production of xenorhabdins in batch culture is the following yeast extract-salts (YS) broth: yeast extract $5gL^{-1}$; $(NH_4)_2SO_4$ $5gL^{-1}$; $MgSO_4.7H_2O$, $0.2gL^{-1}$; KH_2PO_4 $0.5gL^{-1}$ and K_2HPO_4 $0.5gL^{-1}$, pH 6.8.

Such a medium used and found suitable for production of xenorhabdins in continuous culture is the following: glycerol $20gL^{-1}$; yeast Extract $10gL^{-1}$; $(NH_4)_2SO_4$ $20gL^{-1}$; KH_2PO_4 $10gL^{-1}$; $MgSO_4.7H_2O$ $2.5gL^{-1}$; $CaCl_2.2H_2O$ $0.29gL^{-1}$; $FeSO_4.7H_2O$ $27.8mgL^{-1}$; $MnSO_4.H_2O$ $8.45mgL^{-1}$; $ZnSO_4.7H_2O$ $14.4mgL^{-1}$; $CoCl_2.6H_2O$ $0.10mgL^{-1}$: and $CuSO_4.5H_2O$ $0.19mgL^{-1}$.

It is preferred that the batch processes are carried out at temperatures between 23°C and 37°C, ideally between 23°C and 30°C and optimally at 28°C. The batch processes are preferably commenced at a pH of between 4.5 and 8.0, ideally between 6.3 and 7.5 and optimally at 6.8.

The continuous processes of the invention are preferably carried out between 23°C and 37°C, ideally at 28°C. They are preferably carried out at between pH 6.3 and 7.5, and ideally at 6.8. In these processes fresh culture medium is preferably added to give a dilution rate of between $0.01hr^{-1}$ and $0.5hr^{-1}$, most preferably between $0.04hr^{-1}$ and $0.1hr^{-1}$.

The culture media and culture conditions employed in

processes for producing the xenorhabdin compounds of the invention are also suitable for production of the other compounds of the invention having antibiotic and/or pesticidal activity.

The structure of the xenorhabdin compounds of the invention were elucidated by performing an x-ray diffraction analysis on a crystal of the xanthydrol derivative of one homologue, xenorhabdin I. Correlation of mass spectral, ultra violet, nuclear magnetic resonance and retention time data enabled structures to be assigned to the five homologues Xenorhabdin I-V and the acetate of Xenorhabdin II.

The following examples provide detailed descriptions of such procedures, together with a description of fractionation of the isolate and characterization of compounds of the invention.

Example 1

A monoxenic culture of 1^{o} form of **Xenorhadbus nematophilus** strain T319 (Enterobacteriaceae) was cultured on chicken offal as follows: medium (3kg) of 12 parts of homogenised chicken offal to 1 part of polyurethane foam was autoclaved in an evacuated polypropylene bag. The bag was inflated through tubes containing filters (0.45 m) with air. The inoculum was prepared by culturing the **X.nematophilus** in YS broth (500mL) at 28^{o}C for 24 hours. The medium was inoculated and growth proceeded for 5 days.

The inoculated foam-homogenate mixture was steeped and manually mixed in ethanol (2 x 5L) for 10 hours. The combined ethanolic extracts were concentrated by evaporation **in vacuo** at 40^{o}C then lyophilised to yield 143g of extract. This extract (138g) was stirred with ethyl acetate (4 x 500mL) followed by acetone (2 x 500mL), then the organic extracts were decanted, filtered, dried over anhydrous sodium sulfate and finally evaporated to yield a viscous, brown oil (50g).

Fractionation of this oil was guided by an **in vitro** antibacterial bioassay. A solution of the sample was

dissolved in DMSO then applied to a paper disc. This disc was placed on agar previously inoculated with Micrococus luteus. Zones of inhibition were measured after incubation at 37°C for 20 hours.

The oil (50g) was dissolved in petroleum ether (40-60°, 150mL) the Kieselgel 60 (35-70 mesh) (100mL) was added and the solution evaporated. A dry sticky residue was recovered and it was applied to the top of a column (900 x 50mm) of Kieselgel 60 (35-70 mesh, 1790g) which was equilibrated in petroleum ether (40-60°). The column was eluted sequentially as follows with the weight recovered indicated in the parentheses; pet. ether 4L; pet. ether: ethyl acetate 9:1 2L, 3:1 2L (21.1g); 2:1 4L (7.7g); 1:1 2L (1.86g); 1:2 4L (2.65g); ethyl acetate 2L (92g); methanol 4L (9.64g).

The yellow fraction eluted in petroleum ether:ethyl acetate 1:2 displayed antibacterial activity at an M.I.C. of $100g.mL^{-1}$.

The most active fraction (2.65g) was triturated with petroleum ether 40-60° (100mL), filtered then dried to yield 555mg of pale yellow solid.

A solution of this pale yellow solid (80mg) in methanol (4mL) was subjected to gel permeation chromatography on a column (88 x 3.0cm, total volume 442mL) of Sephadex LH-20 in methanol. The column eluate was pumped at $1mL.min^{-1}$ and monitored in a flow cell with a Uvicord SII (LKB) at 280nm and fractionated with a Multirac (LKB). Two symmetrical u.v. absorbances were detected which corresponded to an inactive colourless crystalline solid at 331-373mL (10mg) and a crystalline yellow solid at 433-475mL (57.8mg). Several batches of sample were chromatographed to afford 265mg of yellow solid.

This solid was subjected to preparative, isocratic, reverse phase HPLC on a Whatman Partisil-10 ODS column (10m, 9.4 x 500mm). The eluant (acetonitrile:water 1:1) was delivered at $4mL.min^{-1}$ with a Waters Model 6000A pump and the eluate monitored with a Waters Model 450 variable wavelength detector at 280nm. Injections of 2-5mg of sample

in acetonitrile (200 L) were applied to the column. Three main constituents were detected and evaporation in vacuo followed by lyophilisation afforded three pure yellow, equiactive compounds, xenorhabdins I (42.7mg), II (8.0mg), and III (4.0mg).

Xenorhabdin I: MP 192-193^{o}C.

^{13}C NMR (DMSO d6, 25MHz) 13.98 (q), 21.93 (t), 24.86 (t), 30.88 (t), 34.69 (t), 110.74 (d), 115.42 (s), 133.62 (s), 134.09 (s), 167.96 (s), 171.91 (s) ppm.

^{1}H NMR $(CD_3)_2CO$ 200MHz reference acetone 2.04 ppm; 9.70 (0.37H broad); 8.74 (0.38H, broad); 6.98 (0.64H, sharp s); 3.22 (0.28H s); 2.93 (br s); 2.45 (2H, t, J=7.2Hz); 1.64 (2H, br t of d); 1.32 (4H, m); 0.88 (3H, br d of t).

Signals at 9.70, 8.74, 2.93 ppm are exchangeable with CD_3OD.

IR (Nujol) cm^{-1}: 3250 (br), 1670, 1640, 1470.

Mass Spectrum, electron ionisation, m/z (%): 273 (0.6), 272 (5.6), 271 (7), M^+ 270.0496 (55), $C_{11}H_{14}N_2O_2S_2$ requires 270.04982, 174(24), 173(26), 171.9759(100) $C_5H_4N_2OS_2$ requires 171.9766, 145(5), 143(7), 117(5), 101(5), 99(7), 72(5), 71(18), 57(8), 55(12), 45(15), 43(85).

Ultraviolet spectrum (CH_3OH): 390nm (max), 310nm, 250nm.

Xanthydrol Derivative

A solution of Xenorhabdin I (730µg) in acetic acid (50µL) was added to a solution of Xanthydrol (9-hydroxyxanthene) (3.3mg) in acetic acid (50µL). Crystals formed after standing overnight at room temperature. The mother liquor was syringed out and the crystals washed (2 x 50µL) with cold acetic acid. They were recrystallised from a mixture

of methanol (1mL), acetic acid (50μL), acetonitrile (50μL) at 85°. The structure of these crystals was determined by x-ray diffraction.

Mass Spectrum electron ionisation m/z (%): 464(0.8), 182(22), 181(100), 172(23), 152(9), 86(6), 60(8), 45(9), 44(6), 43(11).

Xenorhabdin II: M.P. 210-213°C

^{1}H NMR $(CD_3)_2CO$ 200MHz reference acetone 2.04 ppm; 9.78 (0.14 H br); 6.96 (0.45H, s); 3.29 (0.47H, s); 2.86 small shoulder;2.83 (3H s); 2.44 (2H, t J=7.4Hz); 1.62 (3H, m); 1.25 (2H, d of t); 0.87 (6H, d J=6.5 Hz).

Signals at 9.78, 2.83 are exchangeable with CD_3OD.

Mass spectrum electron ionisation m/z(%):
M^+ 284 (2) $C_{12}H_{16}N_2O_2S_2$. 174(10), 173(13), 172(100), 143(3), 95(7), 69(60), 55(4), 45(5), 43(28).
M^+284.0659(20) $C_{12}H_{16}N_2O_2S_2$ requires 284.0653, 174(10), 173(13), 171.9771(100) $C_5H_4N_2OS_2$ requires 171.9766, 143(3), 95(7), 69(60), 55(4), 45(5), 43(28).

Ultraviolet spectrum (CH_3OH): 390nm (max), 310nm, 250nm.

Xenorhabdin II Acetate

Pyridine (1mL) was added to a solution of Xenorhabdin II (9.6mg) in acetate anhydride (1mL) and the solution left at room temperature for 50 hr. The solution was lyophilised, the residue was dissolved in acetonitrile (1mL) then subjected to preparative isocrataic HPLC in acetonitrile:water (3:2) on a Whatman Magnum 9 ODS column at 4mL min^{-1}. An essentially quantitative yield of acetate was recovered.

Mass spectrum electron ionisation m/z(%): 328(5), 327 (7),

326.0760 $C_{14}H_{18}N_2O_3S_2$ requires 326.0759, 284 (19), 215(5), 214(39), 174(9), 173(11), 172(100), 171(8), 95(8), 69(9), 55(7), 43(35).

^{1}HNMR ($CDCl_3$) 200mHz $(CH_3)_4$ Si reference: 8.08 (1H, sharp s, olefinic), 7.42 (1H, br s, N-H), 2.66 (3H, sharp s, CH_3CO), 2.36 (2H, t, J=7Hz, CH_2-CO), 1.7 (3H, br m, CH_2), 1.26 (2H, br m, CH_2), 0.91 (6H, d, J=7Hz, geminal methyls).

Xenorhabdin III: M.P. 360°C

^{1}H NMR $(CD_3)_2CO$ reference acetone 2.04 ppm; 9.7(m); 6.86(s); 3.27 (s); 2.86 (small shoulder); 2.83 (s); 2.44 (t,J=7.4Hz); 1.64(m); 1.3(m); 0.86(m).

Mass spectrum electron ionisation m/z(%): 298.0815(32), $C_{13}H_{18}N_2O_2S_2$ requires 298.0810, 174(18), 173(20), 171.9769(100), $C_5H_4N_2OS_2$ requires 171.9766, 143(3), 127(3), 117(3), 101(3), 72(3), 57(32), 45(10), 43(26).
Ultraviolet spectrum (CH_3OH): 390nm (max), 310nm, 250nm.

Retention of the xenorhabdins I, II, IV, V and III on a Brownlee RP-18 column (4.6 x 250mm) pumped at 1.5mL.min^{-1} and detected at 405nm were 4.6, 6.0 6.64, 8.98 and 9.6 minutes respectively.

Xenorhabdin IV M.P. 165°

^{1}HNMR $(CD_3)_2CO$ 200 MHz reference acetone 2.04 ppm; 8.85 (0.2H, broad s, NH); 7.08 (1H, sharp s, olefinic H); 3.28 (3H, sharp s, NMe); 2.43 (2H, t, J = 7Hz, CO-CH_2); 1.64 (2H, M, CH_2); 1.25 (4H, M, CH_2-CH_2); 0.85 (3H, M, CH_3).

Mass spectrum, electron ionisation, m/Z (%):286(3), 285(4),

M^+ 284.0654 (24) $C_{12}H_{16}N_2O_2S_2$ requires 248.0653; 189(1), 188(10), 187(11), 186(100), 185(5), 157(2), 130(1), 101(1), 99(1), 86(3), 71(3), 70(1), 69(1), 55(3), 45(3), 43(17), 42(7), 41(5).

Collision-Induced Dissociation Spectrum of M/z 186: 172(5), 171(35), 170(5), 169(81), 159(7), 158(29), 157(100), 156(5), 154(6), 153(42), 144(26), 143(48), 142(33), 141(20), 131(23), 130(42), 129(6), 128(6), 125(29), 124(8), 117(7), 116(29), 114(8), 113(15), 103(18), 102(33), 101(29), 100(15), 98(20), 87(11), 86(42), 85(14), 84(21), 82(16), 81(25), 72(43), 71(10), 70(11), 69(17), 68(5), 66(5), 65(8), 60(5), 57(6), 55(8), 53(6), 52(6), 45(24), 42(20).

Ultraviolet spectrum (CH_3OH) λ max 390 (ε = 10000); 310nm(ε = 1000), 250nm.

Infrared spectrum (K Br disc) cm^{-1}: 3260, 2920, 1680, 1650, 1610, 1540, 1440, 1240, 830

Xenorhabdin V

1HNMR $(CD_3)_2CO$ 200MHz reference acetone 2.04 ppm; 8.9 (m,NH); 7.10 (1H, sharp s, olefinic H); 3.29 (3H, sharp s, NMe); 2.42 (2H, t, J=7 Hz, CO-CH_2); 1.62 (3H, m,); 1.25 (2H, m); 0.87 (6H, d, J=6.5 Hz).

Mass Spectrum, electron ionisation, M/Z (%): 300(2), 299(4), 298.0807(20) $C_{13}H_{18}N_2O_2S_2$ requires 298.0810, 189(1), 188(10), 187(13), 186(100), 130(1), 101(1), 95(3), 86(3), 69(4), 57(3), 56(1), 55(5), 45(3), 44(1), 43(19), 42(7),
41(9).
Ultraviolet spectrum (CH_3OH): 390nm (max), 310nm, 250nm.

Fermentation techniques may be employed for large scale production of xenorhabdins, as will now be exemplified:

Example 2

X.nematophilus strain T319 was grown in 3.5 litres

of yeast extract-salts (YS) medium pH 6.7 which contained 5g.L^{-1} of yeast extract. A stirred laboratory fermenter was employed. It consisted of a 5 litre glass pipe section with stainless steel end plates. The stirrer, a flat blade turbine impeller was driven at 1425rpm. Air was sparged into the fermenter at 0.5vv^{-1}.min^{-1}, so that the dissolved oxygen concentration was maintained above 50% of air saturation. Chilled water was recirculated through an internal cooling coil and an on-off temperature controller was used to activate two 250 watt heating lamps to maintain the culture temperature at 28^{o}C. A 3% v/v inoculum, which was grown for 20 hours in the yeast extract-salts medium in a shaker-incubator, at 28^{o}, was used. The culture was grown in the fermenter for 48 hours before harvesting. A Beckman J2-21M centrifuge fitted with a continuous flow large pellet core (800mL) rotor operating at 15 000rpm (30 000 x g), 15^{o}C was employed to harvest the cells. Sodium azide (600mg) was added to the supernatant (3 litres) then it was pumped (9.7mL.min^{-1}) through a column of Amberlite XAD-resin (34 x 2.5cm). The resin was washed with water (2.5 litres). Evaporation of the methanolic fraction afforded a residue from which the xenorhabdins could be isolated by the chromatographic procedures outlined above.

Example 3

Xenorhabdus nematophilus strain XQ-1 was grown in a continuous culture. A Braun U-series fermenter with 5L glass vessel was set up for a culture working volume of 2.3L. Temperature was controlled at 28^{o}C, and pH at 7.0 by automatic addition of 20% w/v phosphoric acid or 50% w/w sodium hydroxide as required. The level of dissolved oxygen was maintained at or above 40% of air saturation by manually adjusting air flow rate and stirrer speed.

Medium was prepared in 18L batches containing the following ingredients: yeast extract 15g.L^{-1}; sodium chloride 5g.L^{-1}; ammonium sulfate 1g.L^{-1}; magnesium sulfate 0.5g.L^{-1}; potassium dihydrogen and dipotassium hydrogen orthophosphates each 1g.L^{-1}. After sterilization by autoclaving, 2L of this medium was introduced to the

sterile fermenter and inoculated with 100mL of an overnight culture of the primary form of XQ-1, in yeast extract-salts broth. This was allowed to grow batchwise for 8 hours, then fresh medium was pumped to the fermenter at a rate sufficient to give a dilution rate of $0.05hr^{-1}$. Thus one culture volume was collected in the reservoir via the culture off-take tube every 20 hours. Xenorhabdin production began within 24 hours and reached steady-state concentrations in the whole culture of $10.8mg.L^{-1}$ xenorhabdin I and $36.4mg.L^{-1}$ xenorhabdin II, giving a total productivity of some $2.4mg.L^{-1}.hr^{-1}$. Operation of the fermentation may be continued until equipment failure, productivity loss, or infection occurs. In this case significant reversion of the culture to the non-producing secondary form was observed after 140 hours when xenorhabdin productivity had fallen to 30% of the initial steady-state level, and 2^o forms accounted for 31% of the viable cell population.

The growth, in agar, of the following organisms was found to be inhibited by the presence of an Xenorhabdus isolate according to this invention: Cellulomonas sp, S.aureus, B.cereus subsp. mycoides, B. polymyxa, B.subtilis, E.coli, B.thuringiensis, Sh.sonnei, Serratia sp, Pr.vulgaris, Erw.carotovora, C.albicans and S.cerevisiae.

It is to be understood that antibiotics according to this invention include the antibacterially active salts and other derivatives of the xenorhabdins. Moreover, the invention also embraces pharmaceutical compositions containing the antibiotics, as well as the use of the antibiotics in the treatment or prevention of disease.

The compounds of the invention may be administered orally, parentally or topically in dosage forms such as capsules, boluses, tablets, suppositories, solutions, suspensions, dispersions, powders, dusts, creams and gels.

The formulations will contain at least on of the compounds of the invention and may contain other pharmaceutically active agents.

The formulations in solid form for oral administration

such as tablets, capsules, pills, powders, granules and the like will usually be formulated with finely divided diluents, fillers, disintegrating agents and/or binders such as starch, lactose, talc, magnesium stearate, vegetable gums and the like.

Liquid formulations for oral administration such as syrups, solutions, suspensions, elixirs and the like will usually be formulated with an aqueous base. Parenteral formulations will be, for example sterile solutions, suspensions or emulsions in water or organic solvents.

Topical formulations will include solutions, suspensions, ointments, micronised powders, aerosols and the like.

The formulations may be sterilised and/or may contain adjuvants such as preservatives, stabilizers wetting agents, emulsifiers, salts to vary osmotic pressure, or buffers.

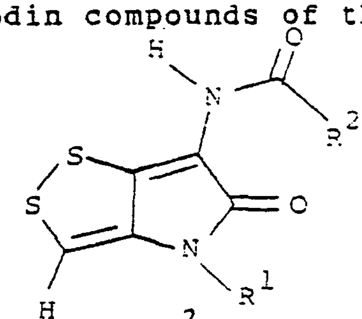
In the pesticidal application of the invention, the compounds of the invention can be used alone or together with suitable carriers and/or diluents. The carriers or diluents can be solid or liquid, such as solvents, dispersing agents, wetting agents, binders, thickeners and extenders.

Pesticidal formulations are produced by conventional means such as mixing and/or grinding with suitable carriers or diluents.

Examples of solid preparations are wettable powders, dusts and granules. Examples of liquid preparations are solutions, water dispersible concentrates, emulsions or pastes.

CLAIMS

1. Xenorhabdin compounds of the formula I:



(I)

wherein R^1 is hydrogen and R^2 is n-pentyl, 4-methylpentyl or n-heptyl; or R^1 is methyl, and R^2 is n-pentyl or 4-methylpentyl; and the salts, acyl and other derivatives thereof.

2. 6-hexanoylamino-1,2-dithiolo[4,3-b]pyrrol-5-(4H)--one or the salts, acyl and other derivatives thereof.

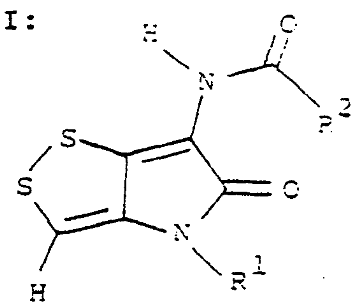
3. 6-(5-methylhexanoylamino)-1,2-dithiolo[4,3-b] pyrrol-5(4H)-one or the salts, acyl and other derivatives thereof.

4. 6-octanoylamino-1,2-dithiolo[4,3-b]pyrrol-5(4H)--one or the salts, acyl and other derivatives thereof.

5. 6-hexanoylamino-4-methyl-1,2-dithiolo[4,3-b]pyrrol-5(4H)-one or the salts, acyl and other derivatives thereof.

6. 6-(3-methylpentanoylamino)-4-methyl-1,2-dithiolo[4,3-b]pyrrol-5(4H)-one or the salts, acyl and other derivatives thereof.

7. A process for the production of xenorhabdin compounds of formula I:



(I)

wherein R^1 is hydrogen and R^2 is n-pentyl, 4-methylpentyl or n-heptyl; or R^1 is methyl and R^2 is n-pentyl or 4-methylpentyl; and the salts, acyl and other derivatives thereof, which process comprises culturing a xenorhabdin producing strain of **Xenorhabdus nematophilus** or **Xenorhabdus luminiscens** in a suitable culture medium in

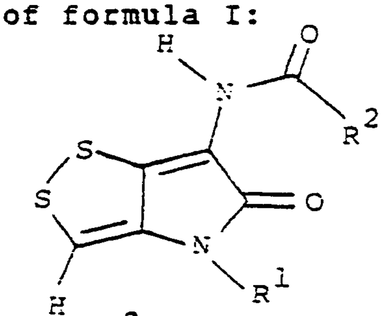
the presence of oxygen, separating said compounds of formula I from the resultant culture broth, and, if desired, converting said compounds of formula I into salts, acyl or other derivatives thereof.

8. The process as defined in claim 7, wherein said culture medium is composed of materials containing suitable carbon and energy sources, suitable nitrogen sources, appropriate quantities of inorganic nutrients, and some source of vitamins and growth factors.

9. The process as defined in claim 7 or claim 8, which process is carried out at a temperature of between 23°C and 37°C.

10. The process as defined in claim 7 or claim 8, which process is carried out at a temperature of 28°C.

11. A continuous process for the production of xenorhabdin compounds of formula I:



(I)

wherein R^1 is hydrogen and R^2 is n-pentyl, 4-methylpentyl or n-heptyl; or R^1 is methyl, and R^2 is n-pentyl or 4-methylpentyl; and the salts, acyl and other derivatives thereof, which process comprises culturing a xenorhabdin producing strain of **Xenorhabdus nematophilus** or **Xenorhabdus luminiscens** in a suitable culture medium in a fermenter in the presence of oxygen, controlling the temperature and pH of the culture in said fermenter, continuously adding fresh culture medium to said fermenter and continuously collecting culture from said fermenter at such a rate to maintain the volume of culture in said fermenter within selected limits and separating said compounds of formula I from the collected culture, and, if desired, converting said compounds of formula I into salts, acyl or other derivatives thereof.

12. The process as defined in claim 11, wherein said

culture medium is composed of materials containing suitable carbon and energy sources, suitable nitrogen sources, appropriate quantities of inorganic nutrients, and some source of vitamins and growth factors.

13. The process as defined in claim 11 or claim 12, wherein said temperature is controlled between 23°C and 37°C.

14. The process as defined in claim 11 or claim 12, wherein said temperature is controlled at 28°C.

15. The process as defined in any one of claims 11 to 14, wherein said pH is controlled between 6.3 and 7.5.

16. The process as defined in any one of claims 11 to 14, wherein said pH is controlled at 6.8.

17. The process as defined in any one of claims 11 to 16, wherein said fresh culture medium is added to give a dilution of between 0.01hr^{-1} and 0.5hr^{-1}.

18. The process as defined in any one of claims 11 to 16, wherein said fresh culture medium is added to give a dilution of between 0.04 hr^{-1} and 0.1hr^{-1}.

19. A process for the preparation of xenorhabdin compounds of formula I as defined in claim 1, said process being substantially as hereinbefore described with reference to any one of the Examples.

20. A process for the production of compounds having antibiotic and/or pesticidal activity and the salts, acyl and other derivatives thereof, not being xenorhabdin compounds of formula I as defined in claim 1, which process comprises culturing a xenorhabdin producing strain of **Xenorhabdus nematophilus or Xenorhabdus luminescens** in a suitable culture medium in the presence of oxygen, separating said compounds having antibiotic and/or pesticidal activity from the resultant culture broth and, if desired, converting said separated compounds into salts, acyl or other derivatives thereof.

21. A continuous process for the production of compounds having antibiotic and/or pesticidal activity and the salts, acyl and other derivatives thereof, not being xenorhabdin compounds of formula I as defined in claim 1,

which process comprises culturing a xenorhabdin producing strain of **Xenorhabdus nematophilus** or **Xenorhabdus luminiscens** in a suitable culture medium in a fermenter in the presence of oxygen, controlling the temperature and pH of the culture in said fermenter, continuously adding fresh culture medium to said fermenter and continuously collecting culture from said fermenter at such a rate to maintain the volume of culture in said fermenter within selected limits and separating said compounds having antibiotic and/or pesticidal activity from the collected culture, and, if desired, converting said separated compounds into salts, acyl or other derivatives thereof.

22. Xenorhabdin compounds of formula I as defined in claim 1, whenever prepared by a process as defined in any one of claims 7 to 19.

23. Compounds having antibiotic and/or pesticidal activity, not being xenorhabdin compounds of formula I as defined in claim 1, whenever prepared by a process as defined in claim 20 or 21.

24. A pharmaceutical formulation which comprises as active ingredient at least one compound as defined in any one of claims 1 to 6, 22 or 23 or a pharmaceutically acceptable salt, acyl or other derivative thereof together with a pharmaceutically acceptable carrier or diluent therefor.

25. A method for the prevention or control of infectious disease in a mammal requiring said prevention or control, which method comprises administering to said mammal an effective amount of at least one compound as defined in any one of claims 1 to 6, 22 or 23 or a pharmaceutically acceptable salt, acyl or other derivative thereof or of a formulation as defined in claim 24.

26. A pesticidal formulation which comprises as active ingredient at least one compound as defined in any on of claims 1 to 6, 22 or 23 or a salt acyl or other derivative thereof, together with a conventional pesticidal carrier or diluent therefor.

27. A method of killing or controlling pests at a

locus at which said pests occur or are expected to occur, which method comprises applying to said locus, an effective amount of at least one compound as defined in any one of claims 1 to 6, 22 or 23 or a salt, acyl or other derivative thereof, or of a formulation as defined in claim 26.

ONE SHEET

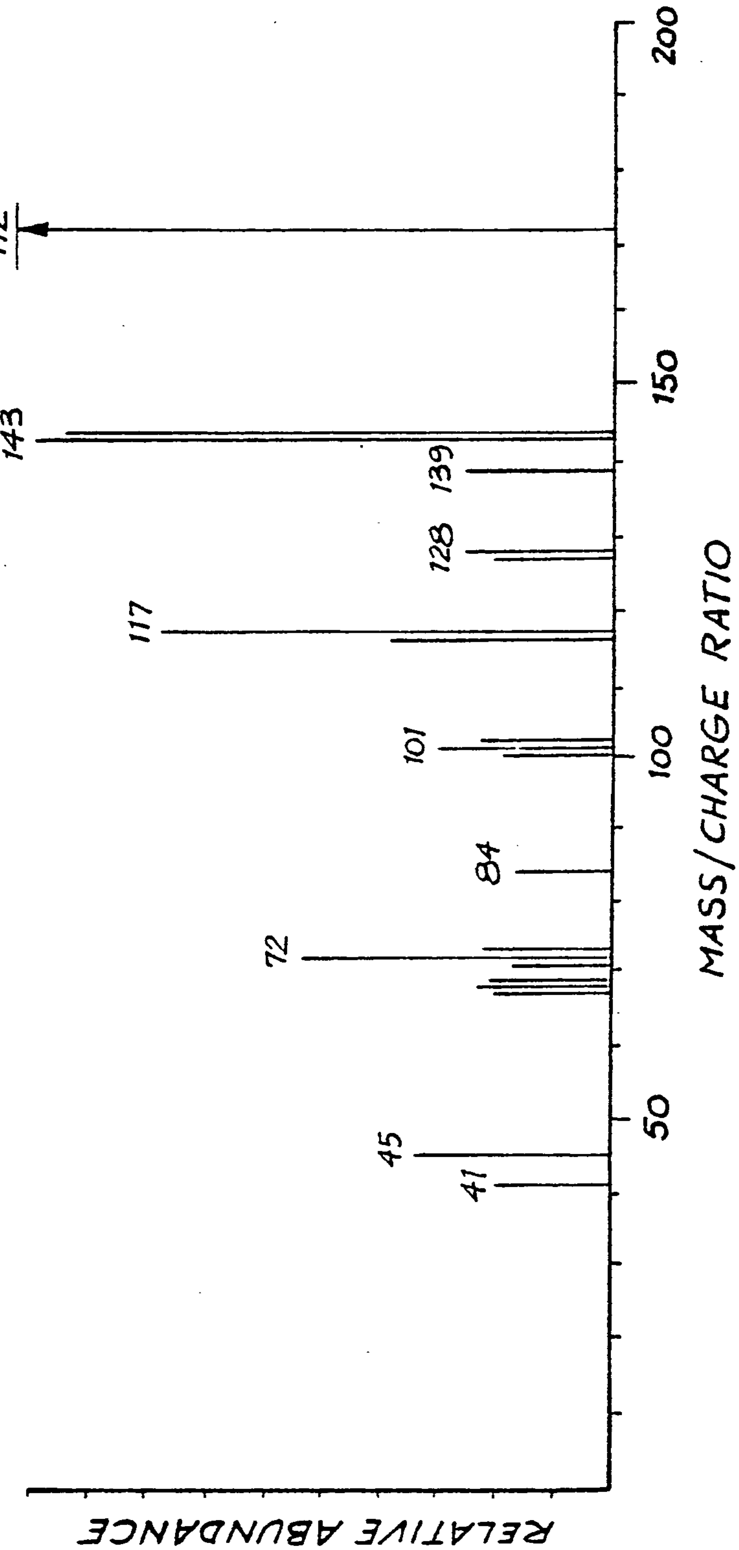


FIG. 1

INTERNATIONAL SEARCH REPORT

International Application No PCT/AU 83/00156

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.^{3} C07D 495/04, C12P 17/18, C12R 1/01.// A61K 31/40, A01N 43/90

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
IPC	C07D 495/04
US Cl.	548/453

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [6]

AU: C07D 495/04, C12P 17/18

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A	AU, B, 50435/59 (263952) (CIBA LTD.) 14 January 1960 (14.01.60) (& US, A, 3014922)	(1)

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
16 December 1983 (16.12.83)	20 December 1983 (20-12-83)
International Searching Authority [1]	**Signature of Authorized Officer** [10]
Australian Patent Office	R.M. Dorning RM Dorning

Form PCT/ISA/210 (second sheet) (October 1981)